2488-Plat
Sensitive Detection of NAD+/NADP+ via Strong Coupling Fluorescence from Silver Nanoclusters
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The pyridine nucleotides (NAD+, NADP+) are the major coenzymes participate in multiple redox processes in living cells. Both NAD+ and NADP+ are not fluorescent and almost structurally identical, so it is difficult to directly distinguish NAD+ or NADP+ via optical methods (such as fluorescence and Raman spectroscopy). We report here a sensitive probe of NAD+/NADP+ based on fluorescent silver nanoclusters with dual emission band. The silver nanoclusters with an initial fluorescent emission peak at 410 nm were synthesized by etching large size silver nanoparticles. With the addition of NAD+/NADP+ solution, due to the strong coupling (charge-transfer) between silver nanoclusters and ligands (NAD+/NADP+), a new fluorescence emission peak of the silver nanoclusters was found and raised at 550 nm and the fluorescence intensity was dependent on the ratio between NAD+ and NADP+. The time-resolved fluorescence decay (at 550 nm) of silver nanoclusters showed a single-exponential decay lifetime of 3.9 ns caused by the strong coupling between silver nanoclusters and ligands (NAD+/NADP+). Meanwhile, the 410 nm emission was also selectively enhanced by the different ratio of NAD+ /NADP+ molecules. The intensity ratio of fluorescence emission at 410nm and 550nm may be useful to monitor the levels of NAD+ /NADP+ in aqueous solutions, cellular extracts and living cells. Candidate mechanisms and the analysis of time resolved emission spectra will be discussed.

2489-Plat
Non-Destructive Label-Free Monitoring of Drug Intake in Live Cells using ATR FT-IR Spectroscopy
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Drug resistance is one of the major challenges in treatment of cancer. The study of the intake of drugs by cancer cells therefore is vital for elucidating the mechanism of drug resistance. Currently to quantify the intake of drugs involve destructive methods such as lysing the cell at each time point of analysis followed by UV-Vis or fluorescence measurements. With this approach, different batches of cell are used for each time point, increasing the biological variability, and it is not possible to perform further analysis on the same cell sample. Attenuated total reflection Fourier transform infrared (ATR FT-IR) is a promising non-destructive label-free while chemically specific technique for analysis of biomedical samples. In this work we demonstrated the quantification of drugs (e.g. doxorubicin), in situ, using the ATR FT-IR method to obtain the drug diffusion profile in the live cell. HeLa cells grown on a multi-bounce ATR crystal were treated with 20 micromolar of doxorubicin, a concentration level that is relevant to cancer studies, and FT-IR spectra were collected in a time course using a MCT detector. Quantification of doxorubicin in the cell was made using the signature peak at 1284 cm-1 showing the accumulation of the drug in cells as a function of time. The results from the ATR FT-IR measurements have shown that the doxorubicin concentration in the living cell increases from 0 to >50 micromolar after 2 hours of treatment demonstrating the partitioning effect of the drug in the cell. Furthermore, the cells also present a signature spectrum, which allows to follow the cell viability in parallel with the drug intake. This is a fast, cost-effective and chemically selective method which does not require sophisticated equipment and can be adapted easily.

2490-Plat
Photobleaching Correction in Fluorescence Correlation Spectroscopy
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Fluorescence correlation spectroscopy (FCS) is a fluorescence technique conventionally used to study the kinetics of fluorescent molecules in a dilute solution. Being a non-invasive technique, FCS is now finding wider applications in the study of more complex systems like the dynamics of DNA or proteins in living cells and cell membranes. Unlike an ordinary dye solution, the dynamics of macromolecules like proteins or entangled DNA, in crowded environments is often slow and subdiffusive in nature. This in turn leads to longer residence times of the attached fluorophores in the excitation volume of the microscope. As a result, photobleaching becomes a problem: the number of photons available to calculate the intensity autocorrelation function is limited, and the decay of the fluorescence intensity itself contributes a spurious signal to the autocorrelation function that can easily obscure the signature of the molecular dynamics of interest.
We present a method of calculating the intensity autocorrelation function from the arrival times of the photons on the detector that maximizes the information content while correcting for the effect of photobleaching to yield an autocorrelation function that reflects only the underlying dynamics of the sample. For this purpose, we determine the overall photobleaching rate by fitting a multi-exponential decay to the fluorescence intensity. Then we assign a weight to each photon using the reciprocal of this fit function. The autocorrelation function is then calculated from all photon pairs using the product of the individual photon weights. This gives late-arriving fluorescence photons that come from a partially photobleached sample a higher weight than those photons that arrived earlier, compensating for the loss of some of the fluorophores. We demonstrate the utility of this technique by acquiring artifact-free FCS data from entangled DNA solutions and from chromosomal DNA in E. coli.

Platform: Cell Mechanics, Mechanosensing, and Motility II

2491-Plat
High Resolution, Large Deformation 3D Traction Force Microscopy
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Traction force microscopy (TFM) is a powerful approach of quantifying cell-material interactions, which over the last two decades has contributed significantly to our understanding of cellular mechanosensing and mechanotransduction. In addition, recent advances in three-dimensional (3D) imaging and traction force analysis (3D TFM) have highlighted the significance of the third dimension in influencing various cellular processes. Yet irrespective of dimensionality almost all TFM approaches have relied on a linear elastic theory framework to calculate cell surface tractions.
This talk presents a new high-resolution 3D TFM algorithm, which utilizes a large deformation formulation to quantify cellular displacement fields with unprecedented resolution. The results feature some of the first experimental evidence that cells are indeed capable of exerting large material deformations, which require the formulation of a new theoretical TFM framework to accurately calculate traction forces. Based on our previous 3D TFM technique we reformulate our approach to accurately account for large material deformation and quantitatively contrast and compare both linear and large deformation frameworks as a function of the applied cell deformation. Particular attention is paid in estimating the accuracy penalty associated with utilizing a traditional linear elastic approach in the presence of large deformation gradients.

2492-Plat
Correlative Traction Force Microscopy and Fluorescence Fluctuation Analysis of Molecular Aggregation and Complex Formation in Cell Adhesions in Distinct Microenvironments
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The mechanical properties of the cellular microenvironment regulate processes that include migration, proliferation, and differentiation. These interactions occur at adhesions, which serve as both traction points and signaling hubs and mediate bi-directional sensing and responses to specific features of the surrounding extracellular matrix (ECM). Adhesions execute these activities through an intricate network of putative molecular interactions that largely remain to be demonstrated and characterized functionally in living cells. The challenge is to capture the highly localized and transient associations that characterize these activities in adhesions and determine how they respond to different microenvironments. In this study, we use high-resolution fluorescence fluctuation microscopy to map the formation and stoichiometry of integrin-associated complexes in the adhesions that populate the leading edge of migrating cells. We focus on putative integrin activating (kindlin and talin) and actin-linking (talin, vinculin and a-actinin) molecules and show that all molecules are present in adhesions as soon as they are visible; however, they form integrin containing